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TITLE: The Hygiene Hypothesis and Breast Cancer: A Novel Application of an Etiologic Theory for Allergies, Asthma, and Other Immune Disorders

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14. ABSTRACT
The "hygiene hypothesis", the idea that reduced exposure to important microbes, especially in childhood, impacts development of asthma and allergies, may have application to breast cancer. This research project aims to explore the hygiene hypothesis as it might relate to breast cancer development, thereby assessing its utility for more comprehensive future research. This research project aimed to interview a population-based series of Californian women recently diagnosed with breast cancer and a matched set of healthy control women as regards age-specific experiences relevant to microbial exposures. This project is currently at the end of Year 3, with an approved one-year no-cost extension. To date, we have interviewed over 350 study subjects and carried new control ascertainment procedures using mailing list sampling methods. Of n=743 cases identified, we have successfully interviewed 51% (n=379). 9% were deemed ineligible to participate on the basis of being deceased, ill or unable to communicate due to senility or language. Regardless, proportions of cases refusing to participate in the study are higher than our original estimates, including 19% "hard-refusing" and 31% passively or soft refusing to participate.

15. SUBJECT TERMS
Immunologic exposures, infectious exposures, early life exposures, socioeconomic status, population-based, multiethnic, case-control study

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
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Introduction

Breast cancer is the most commonly diagnosed cancer in women worldwide, and incidence and mortality rates have increased substantially over the past 50 years. Reasons for these increases are not entirely clear, because breast cancer causes remain incompletely understood. In the absence of means of primary prevention for breast cancer, partial understanding of its causation compels research into new etiologic hypotheses. Identification of novel hypotheses with promise for detailed etiologic investigation should take into consideration the established features of breast cancer epidemiology. A group of factors meeting these criteria are those mediating reduced or delayed exposure to microbes, especially in childhood. Microbial exposures are thought to influence the development of a robust immune system and have been well studied in the etiologies of allergies, asthma, autoimmune disease and other disorders of immune function. This research has led to the "hygiene hypothesis", the idea that reduced or delayed exposures to important microbial inputs hamper the development of a healthy immune system in early life and the maintenance of such a system in adult life, which in turn increases vulnerability to the development of chronic diseases. This research project aims to flesh out the hygiene hypothesis as it might relate to breast cancer development, thereby assessing its utility for more comprehensive future research. We originally planned to interview a population-based series of 500 Californian women recently diagnosed with breast cancer and 500 healthy control women as regards age-specific experiences relevant to microbial exposures.

Body

At the end of Year 3, we had accomplished most of the tasks as laid out in our approved Statement of Work. However, tasks involving subject interviewing and data collection were delayed after longer-than-expected time receiving human subjects protection approvals in Year 1. Below, we provide detail on each task in the Statement of Work and its status.

Task 1. Develop structured questionnaire, including appropriate questions about microbial exposures for breast cancer by translating concepts from existing hygiene hypothesis literature and incorporating questions from questionnaires from breast cancer studies and a Hodgkin's disease study (months 1-3)

a. Compile comprehensive list of topics from hygiene hypothesis literature

We carefully reviewed the hygiene hypothesis literature and compiled a comprehensive list of topics to be included in the questionnaire (Year 1).

b. Obtain language for some questions from Dr. Liu and other authors

We obtained language for some questions from Dr. Liu and other authors, e.g. allergy section of the questionnaire (Year 1).

c. Pilot test for appropriateness for women aged 50 and older

We tested the questionnaire for research question appropriateness for women aged 50 and older. This entailed consideration of cohort-specific experiences in formulating and editing questions (Year 1).

d. Pilot test for variation in concept by ethnicity

We pilot tested the questionnaires among women of white, Asian, and Hispanic ethnicities and incorporated input from them regarding answer choices and terminology. For example, we added different housing types to a question about childhood housing when an Asian woman responded that she grew up in a barracks (Year 1).

e. Pilot test reliability when asked of same person

We have tested reliability when asked of same person by testing slightly revised versions of the questionnaire on the same women, then comparing the answers to see if the answer was comparable.

Task 2. Finalize study documents, obtain needed approvals and complete other preparations for study commencement (months 4-9)

a. Finalize questionnaire and verbal consent scripts

The final questionnaires (Appendix A) and consent form in English (Appendix B) and Spanish (Appendix C) are attached.

b. Translate questionnaire to Spanish

The questionnaire and other relevant study documents were translated into Spanish (Appendix D)

c. Obtain Institutional Review Board approvals

After many months of communications, we received final approvals for this research project from the NCCC Institutional Review Board on 9/11/07 and from the HSRRB on 7/26/06. Both of these approval documents are included in Appendix E.

d. Create study tracking system

In Year 1, we created the study tracking system using Microsoft Access software. The system includes capacity for Computer Assisted Telephone Interviewing (CATI) to improve telephone interviewers' efficiency in data collection. In Year 2, this system was updated to include capacity for Spanish language interviewing and control subject frequency-matching.

e. Hire and train interviewers

In Year 2, we hired and trained 3 interviewers, one of whom is bilingual in Spanish. In Year 3, 2 of these interviewers continued to work on the study .

Task 3. Recruit a selection of women recently diagnosed with invasive breast cancer, and age-and race-matched healthy women and interview them about hygiene-hypothesis-relevant exposures as well as established breast cancer risk factors, using study questionnaire (months 9-27)

We originally planned to begin interviewing subjects by month 9 of Year 1 (March 2006). However, it took longer than we anticipated receiving NCCC IRB and HSRRB clearances. Upon completion of the pilot testing and planning process, we made several changes to the statement of work from its original form, include reducing sample size from 1050 women to 1000, modifying the means of control selection from random-digit dialing to an address-based sampling procedure, dropping the life calendar from the subject mailing, and including a saliva specimen retrieval kit with the mailing, as described below. Dr. Carole Christian, our Army Contracting Officer Representative, confirmed via email on 5/30/06 that these changes were not significant enough to warrant a formal change of SOW.

a. Obtain listing of eligible cases from population-based Greater Bay Area Cancer Registry

We obtained all relevant approvals and clearances from the Greater Bay Area Cancer Registry and have received listings of all eligible cases. In light of the unexpected delays in obtaining human subjects approvals as described above, we altered the dates of diagnosis from our original proposal in order to have the greatest chances of contacting and interviewing patients recently diagnosed with breast cancer. Instead of trying to recruit women diagnosed 1/1/2003-7/30/2004, we instead requested listings for women diagnosed between 10/1/2004 and 9/31/2005. By the end of Year 3, we had received listings for 743 breast cancer patients meeting our age and residency requirements.

b. Establish random-digit dialing (RDD) procedures to ascertain control subjects and conduct RDD

After consulting with study collaborators, particularly co-investigator Dr. Pamela Horn-Ross who is experienced in the design and conduct of breast cancer case-control studies and RDD, we decided to modify the methodology used to ascertain control subjects using a novel, address-based sampling procedure. This procedure follows many of the principles of traditional RDD but has the additional advantage of allowing for mail, telephone, and personal modes of recruitment. In addition, it provides a known sampling frame, which is no longer possible with RDD. This methodology is described in detail in the study protocol, but is summarized briefly here. In February 2007, we purchased a "saturation list" mailing address list from Marketing Systems Group. The list represented a n=10,000 random sample of all US mail-deliverable addresses for San Mateo, Santa Cruz, San Benito, and Monterey counties. Using mailing lists based on residency offers a way to sample the same general population from which the breast cancer cases occur, a fundamental principle of control selection. Introductory letters containing $1 bills were mailed to each address selected. These letters request that recipients call a toll-free line or use email to enumerate their household. Women meeting our selection criteria (female sex, aged 50-79, no prior history of breast cancer) were frequency-matched to cases on five-year age group and race/ethnicity. For households that do not respond to one of the modes within a two-week time frame, we utilized Internet search databases to try and identify a working phone number for the candidate control address. For candidate control addresses for which we can find a phone number (estimated to be 50%), a trained interviewer telephone to attempt to recruit eligible women. If we are unable to contact the household in this way or with additional mailed requests for contact, depending on budgetary constraints, we may send study staff to the physical address to discuss the study personally.

We initiated control ascertainment in April 2007 and interviews are ongoing at the end of Year 3 as we start our one-year no-cost extension. To date, we have identified and interviewed 310 eligible control subjects, with interviews ongoing.

c. Mail letters to physicians to ascertain contraindications to contact

We sent letters to physicians prior to contacting all patients (Years 1-3).

d. Mail letters of invitation to subjects

At the end of Year 3, we had sent letters of invitation to 743 breast cancer patients and to 5500 potential control households.

e. Telephone subjects to confirm participation

At the end of Year 3, our interviewers had called 743 breast cancer patients and 3100 potential control households to invite eligible women to participate.

f. Mail life calendar and informed consent guide to subjects

We learned from pilot testing the pre-interview that a life calendar substantially slowed the pace of the interview and did not seem to substantially aid subject recall, thus we decided to drop it from the pre-interview packet to be mailed to

subjects. We also decided to include with the informed consent documentation in the pre-interview packet a saliva specimen to be mailed back by each participant.

g. Interview subjects by telephone

At the end of Year 3, our interviewers had completed interviews with 689 subjects (n=379 cases and n=310 controls) by telephone, with additional women in process of being contacted, searched or scheduled. Regardless, at this time, response rates appear to be considerably lower than we had anticipated.

<u>Case participation rates:</u> Of n=743 cases identified, we have successfully interviewed 51% (n=379). 9% of cases (n=66) were deemed ineligible on the basis of being deceased (n=20), too ill (n=12), not speaking fluent English or Spanish (n=24), comprehension problems/senility (n=8), or doctor disapproval of contact (n=2). However, the numbers of women refusing to participate in the study are higher than we would have anticipated, including 3.5% using the opt-out box on the initial response form (n=26) and 15% of cases "hard-refusing" (n=109) on telephone contact. An additional 2% of cases (n=15) refused to participate because of their concerns about other DOD-funded non-research activities. 101 women have "soft-refused" or "passively refused", meaning they have not responded to repeated mail and phone contact.

<u>Control participation rates:</u> Of the 5500 controls contacted, 47% of controls (n=2582) were deemed ineligible on the basis of having had breast cancer (n=51), too ill (n=4), not speaking fluent English or Spanish (n=45), comprehension problems/senility (n=19), no eligible household member (n=1042), unable to determine eligibility (n=996), or address undeliverable or unable to locate new number (n=425). Of the controls that were eligible, we have successfully interviewed 6% (n=310). 15% of controls "hard-refusing" (n=292) on telephone contact. An additional 0.3% of controls (n=14) refused to participate because of their concerns about other DOD-funded non-research activities. 2056 (37%) women have "soft-refused" or who have not responded to repeated mail and phone contact will be targeted for further requests to participate the remaining month of data collection.

h. Send subjects thank you note and compensation

We send thank you letters along with the compensation at the completion of the interview to all participants. By the end of Year 3, we had sent 683 thank you notes and checks or gift cards.

i. Call back subjects to resolve discrepancies

When necessary, interviewers are calling subjects back to resolve discrepancies.

j. Enter and clean data to create analytic database

Preliminary data cleaning and variable consistency checks have been initiated and will continue as subject interviews draw to a close during our no-cost extension year.

Task 4. Preliminary analyses: Evaluate whether risk of breast cancer diagnosed in women aged 50 years or older is associated with "hygiene hypothesis"-relevant exposures

independently of reproductive characteristics and other established BC risk factors (Specific Aim 1) and assess whether associations could be limited to select demographic or tumor groups (Months 28-32)

a. Compare distributions of these characteristics between cases and controls

We are still recruiting controls and as such have not yet begun statistical analyses.

b. Estimate relative risk by calculating odds ratios for suggestively associated risk factors

c. Adjust these associations for possible confounders

d. Explore possible effect modification by race/ethnicity and tumor characteristics

e. Assess selection bias and consider influence on results

Key Research Accomplishments

At the end of Year 3, we had not completed data collection procedures (see above) so we have no key research accomplishments yet. Dr. Clarke will be presenting preliminary data as a poster at the DOD Era of Hope meeting in Baltimore in June, 2008.

Reportable Outcomes

At the end of Year 3, there were no reportable outcomes resulting from these research activities, including manuscripts, patents, or other scientific products.

Conclusion

As we have yet to complete data collection, we do not have any research findings at this time to summarize and interpret.

References

There are no references at this point.

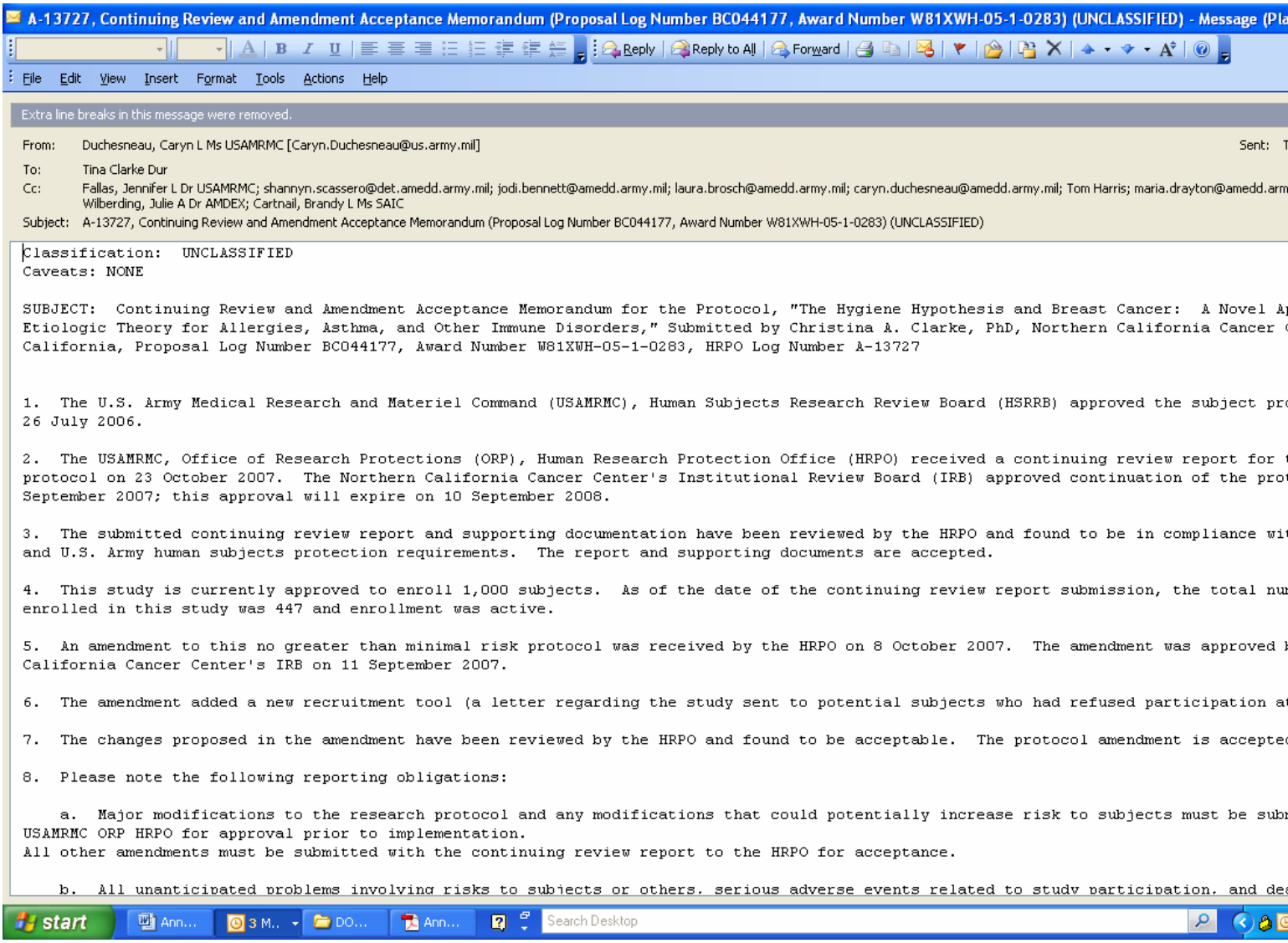
Appendices

Appendix A – Current NCCC and HSRRB Institutional Review Board approvals

Supporting Data

Not applicable.

APPENDIX A: CURRENT HSRRB APPROVAL



A-13727, Continuing Review and Amendment Acceptance Memorandum (Proposal Log Number BC044177, Award Number W81XWH-05-1-0283) (UNCLASSIFIED) - Message (Pla

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From: Duchesneau, Caryn L Ms USAMRMC [Caryn.Duchesneau@us.army.mil] Sent: T

To: Tina Clarke Dur

Cc: Fallas, Jennifer L Dr USAMRMC; shannyn.scassero@det.amedd.army.mil; jodi.bennett@amedd.army.mil; laura.brosch@amedd.army.mil; caryn.duchesneau@amedd.army.mil; Tom Harris; maria.drayton@amedd.arm
Wilberding, Julie A Dr AMDEX; Cartnail, Brandy L Ms SAIC

Subject: A-13727, Continuing Review and Amendment Acceptance Memorandum (Proposal Log Number BC044177, Award Number W81XWH-05-1-0283) (UNCLASSIFIED)

Classification: UNCLASSIFIED
Caveats: NONE

SUBJECT: Continuing Review and Amendment Acceptance Memorandum for the Protocol, "The Hygiene Hypothesis and Breast Cancer: A Novel Ap
Etiologic Theory for Allergies, Asthma, and Other Immune Disorders," Submitted by Christina A. Clarke, PhD, Northern California Cancer C
California, Proposal Log Number BC044177, Award Number W81XWH-05-1-0283, HRPO Log Number A-13727

1. The U.S. Army Medical Research and Materiel Command (USAMRMC), Human Subjects Research Review Board (HSRRB) approved the subject pro
26 July 2006.

2. The USAMRMC, Office of Research Protections (ORP), Human Research Protection Office (HRPO) received a continuing review report for t
protocol on 23 October 2007. The Northern California Cancer Center's Institutional Review Board (IRB) approved continuation of the prot
September 2007; this approval will expire on 10 September 2008.

3. The submitted continuing review report and supporting documentation have been reviewed by the HRPO and found to be in compliance wit
and U.S. Army human subjects protection requirements. The report and supporting documents are accepted.

4. This study is currently approved to enroll 1,000 subjects. As of the date of the continuing review report submission, the total nur
enrolled in this study was 447 and enrollment was active.

5. An amendment to this no greater than minimal risk protocol was received by the HRPO on 8 October 2007. The amendment was approved b
California Cancer Center's IRB on 11 September 2007.

6. The amendment added a new recruitment tool (a letter regarding the study sent to potential subjects who had refused participation at

7. The changes proposed in the amendment have been reviewed by the HRPO and found to be acceptable. The protocol amendment is accepted

8. Please note the following reporting obligations:

a. Major modifications to the research protocol and any modifications that could potentially increase risk to subjects must be subm
USAMRMC ORP HRPO for approval prior to implementation.
All other amendments must be submitted with the continuing review report to the HRPO for acceptance.

b. All unanticipated problems involving risks to subjects or others, serious adverse events related to study participation, and de

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NORTHERN CALIFORNIA CANCER CENTER

2201 Walnut Avenue, Suite 300, Fremont, CA 94538 - (510) 608-5000 - FAX (510) 608-5095

INSTITUTIONAL REVIEW BOARD
MEMO OF ACTION TAKEN

TO: Christina Clarke, Ph.D. **DATE:** October 23, 2007

FROM: Rob McLaughlin
Chair, Institutional Review Board

SUBJECT: 2005-004 The Hygiene Hypothesis and Breast Cancer: A Novel Application of an Etiologic Theory for Allergies, Asthma and Other Immune Disorders
Date of Administrative Board Review: 10/23/2007
Application for Modification

Any Modification to the study that affects the participation of human subjects must receive prior approval from the IRB.

Any complications related to subject participation; including adverse drug reactions and subject complaints must be reported immediately to the IRB. Please submit this information to the Legal and Regulatory Affairs Officer.

The Institutional Review Board (IRB) of the Northern California Cancer Center has reviewed the above referenced research project and have made the following determination:

The modification of Appendix N to delete the household enumeration box was approved and archived to the IRB file as requested.

The protocol number 2005-004 must be used on all applications and correspondence related to this study.

Project approval will expire on September 10, 2008. If the project is to continue beyond that date, it must be reviewed not less than on an annual basis and in accordance with the Cancer Center's Federal-Wide Assurance (FWA00005005).

cc: IRB file